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Single exposure of human fibroblasts (WI-38) to a sub-cytotoxic dose of UVB induces premature senescence

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Abstract In this work, we present a new model of stress-induced premature senescence obtained by exposing human fibroblasts (WI-38) at early passages (passages 2–4) to a single sub-cytotoxic dose of UVB (200 mJ/cm^2). We show that this treatment leads to the appearance of several biomarkers of senescence such as enlarged and flattened cell morphology, the presence of nuclear heterochromatic foci and β-galactosidase activity. Furthermore, we demonstrate that a mild ROS production and p53 activation are upstream events required for the induction of premature senescence. Our method represents an alternative in vitro model in photoaging research and could be used to test potential anti-photoaging compounds.

Keywords: Premature senescence; Oxidative stress; UVB

1. Introduction

Human diploid fibroblasts undergo a limited number of population doublings before entering a state of permanent growth arrest, referred to as replicative senescence or cellular senescence [1,2]. In addition to replicative senescence, cells can also be induced to become senescent prematurely by oxidative stress, DNA damaging agents, histone deacetylase inhibitors, or certain oncogene overexpression [3,4]. Cells in replicative senescence share common features with cells in premature senescence, including for example β-galactosidase activity [5–7].

UVB (290–320 nm) is an inherent component of sunlight that crosses the epidermis, reaching the upper dermis composed mainly of fibroblasts and extracellular matrix [8]. Ultraviolet B radiation (UVB) interacts with cellular chromatophores and photosensitizers resulting in the generation of reactive oxygen species (ROS), DNA damage and activation of signaling pathways related to growth, differentiation, senescence and connective tissue degradation [9]. In this work, we present a new and robust model of stress-induced premature senescence obtained by exposing human fibroblasts (WI-38) at early passages (passages 2–4) to a single sub-cytotoxic dose of UVB (200 mJ/cm^2).

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Abbreviations: GSH, reduced glutathione; IVM, intensified charge-coupled device video microscopy; P-FAK, phosphorilated focal adhesion kinase; PSFs, prematurely senescent fibroblasts; ROS, reactive oxygen species; UVB, ultraviolet B radiation

2. Materials and methods

2.1. Cell cultures

Lung diploid human fibroblasts WI-38 (#CCL-75), purchased from the American Type Culture Collection (USA), were maintained in RPMI medium (GIBCO-Invitrogen, MI, Italy) supplemented with 10% fetal calf serum, 1% non-essential amino acids, 100 U/ml penicillin and 100 ng/ml streptomycin. Cells were seeded at density of 2×10^5 cells in the Petri dishes and maintained at 37 °C. Cells at passages 2–4 were used for study. For immunofluorescence analysis, cells were seeded on 13-mm diameter glass cover-slips in separate wells.

2.2. UVB light exposure

Exposure to a single dose of UVB (200 mJ/cm^2) was obtained as follows: 24 h after plating, the cells were exposed to UVB radiation in a thin layer of PBS (pH 7.4) using one Philips TL 20 W/12 lamp (Philips, The Netherlands) which was placed 10 cm above the flasks. In order to eliminate UV radiations not in the UVB range, a Kodak filter (type Kodacell TL 401) was used. In these conditions the UVB radiant flux density was measured with an Osram Centra radiometer. The radiometric measurements were performed for each experiment. This procedure has been described elsewhere [10,11]. Control samples were submitted to the same conditions without UVB radiation.

2.3. Scanning electron microscopy (SEM)

Cells were fixed with 2.5% glutaraldehyde in 0.1 M cacodylate buffer (pH 7.4) at room temperature for 20 min. Following post-fixation in 1% OsO4 for 30 min, these cells were then dehydrated through graded ethanol, critical point-dried in CO_2 and gold-coated by sputtering by Balzers Union SCD 040 apparatus. The samples were examined with a Cambridge 360 scanning electron microscope.

2.4. Cell growth and cell cycle analysis

Cell proliferation was analyzed by performing growth curves both in control and UVB irradiated WI-38 fibroblasts. Cells were harvested daily and their number was determined by counting cells using the Trypan blue (GIBCO) exclusion test. This procedure was performed for a total of 6 days. Quantitative results reported in the text were expressed as percentage decrease of the cell number in irradiated versus non-irradiated samples.

For DNA analysis, ethanol-fixed cells were incubated with propidium iodide (40 μg/ml, Sigma) and RNAse (10 μM, Sigma) for 45 min at 37 °C. Cell cycle analyses were conducted at different time points after UVB irradiation: 24 h, 48 h, 72 h 96 h and 5 days. Sample analysis was performed on a FACScan flow cytometer by using the Cell Quest software (Becton & Dickinson, Mountain View, CA, USA). At least 20000 events for each sample were statistically analyzed by ModFIT software

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for Macintosh to determine the percentage of cells in G0/G1, S and G2/M phases, respectively. Results are reported as the mean values among three independent experiments performed in triplicate ± standard deviation.

2.5. β-Galactosidase activity

For senescence marker analysis, control and UVB-exposed cells were stained with senescence detection kit (Medical & Biological Laboratories Co., Ltd., Nagoya, Japan) that is designed to histochemically detect β-galactosidase activity in cultured cells. The number of β-galactosidase positive fibroblasts was evaluated at different time points after UVB exposure (24, 48 and 72 h).

2.6. Analytical cytology

For fluorescence microscopy and flow cytometry analyses, cells were fixed with 4% paraformaldehyde and permeabilized with 0.5 Triton X-100 (Sigma). For actin detection cells were stained with fluorescein-phalloidin (Sigma) at 37 °C for 30 min. For phosphorylated focal adhesion kinase (P-FAK, Chemicon, Tecumala CA, USA) and Bcl-2 (Santa Cruz Biotechnology) monoclonal or polyclonal antibodies directed against these antigens were used. After 30 min at 37 °C, cells were washed and then incubated with FITC-labeled anti-mouse or anti-rabbit (Sigma) antibody. For p53 detection, cells were fixed in acetone/methanol 1/1 (v/v) incubated at room temperature with the mouse monoclonal antibodies to p53 (Chemicon) and after three washes in PBS, incubated with FITC-labeled anti-mouse (Sigma) antibody. Morphometric analyses were also performed to evaluate p53 nuclear translocation by counting 300 cells at high magnification (500×).

For a qualitative analysis all samples were mounted on glass coverslips with glycerol-PBS (2:1) and analyzed by intensified charge-coupled device video microscopy (IVM) with a Nikon Microphot fluorescence microscope equipped with a Zeiss CCD camera [12,13]. To analyze the nuclei and to evaluate apoptosis, cells were stained with Hoechst 33258 (Sigma) at 37 °C for 30 min as previously described [14]. Regarding flow cytometry analyses, all the samples were recorded with a FACScan flow cytometer (Becton & Dickinson) equipped with a 488 nm argon laser. A least 20000 events have been acquired. The median values of fluorescence intensity histograms were used to provide a semi-quantitative analysis.

2.7. Evaluation of redox state

Cells (5×10^5) were incubated in 490 μl of Hanks' balanced salt solution (HBSS, pH 7.4) with 5 μl of dihydroethidium (DHE) or dihydrorhodamine 123 (DHR 123) in polypropylene test tubes for 5 min at 37 °C. The final concentration of DHE and DHR 123 were 1 and 10 μmol/L, respectively [15]. Monochlorobimane (Molecular Probes) staining was performed for intracellular reduced glutathione (GSH) detection [16]. Samples were analyzed by a LRS II cytometer (Becton & Dickinson, San Jose', CA, USA) equipped with a 488 argon laser (for DHR123 and DHE detection) and a UVB laser (for monochlorobimane detection).

The median values of fluorescence intensity histograms were used to provide semi-quantitative assessment of GSH content and reactive oxygen species (ROS) production. The fluorescence signal of control cells was set to 100% and the expression levels of GSH content or ROS production in treated cells were reported relative to this.

2.8. Protein extraction and Western blot analysis

Cells were lysed in boiled sample buffer 1 × (50 mM Tris–HCl pH 6.8, 2% SDS, 10% glycerol, 100 mM DTT). In total, 25 μg of total protein extracts were resolved on 12% SDS–PAGE and electrically transferred onto nitrocellulose membranes. Membranes were blocked with TBS-T (20 mM Tris–HC, pH 7.4, 150 mM NaCl, 0.02% Tween-20) containing 5% skimmed milk (Bio-Rad, CA, USA), for 30 min at room

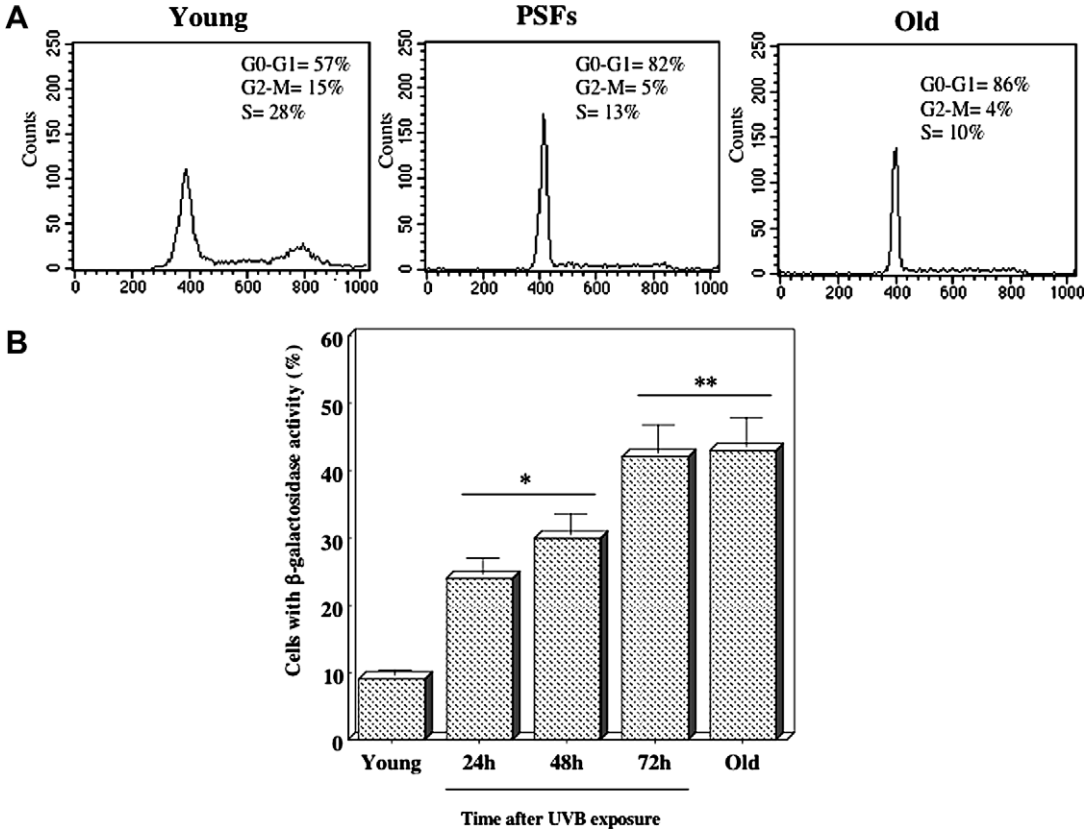

Fig. 1. (A) Cell cycle analysis after PI staining. Analysis of DNA content in control WI-38 fibroblasts (left panel), in prematurely senescent fibroblasts (PSFs) 48 h after UVB exposure (middle panel) and in old fibroblasts (right panel). To note the increase of cells in G0–G1 phase in irradiated fibroblasts with respect to control cells. One representative experiment out of four is shown. (B) Percentage of cells positive to the β-galactosidase activity test. The values reported are the means ± S.E. of four separate experiments. Student's *t*-test to compare control cells, prematurely senescent fibroblasts (PSFs) and old cells was used. (*) Indicates $P < 0.05$ while (**) $P < 0.01$.

temperature, and then incubated overnight at 4 °C with primary antibodies. The following antibodies were used: (i) monoclonal antibody to phosphorylated focal adhesion kinase (FAK) (Chemicon), p53 and α-tubulin (Santa Cruz Biotechnology) and (ii) polyclonal antibody to Bcl-2 (Santa Cruz Biotechnology). Detection was achieved using HRP-conjugated secondary monoclonal or polyclonal antibodies and by ECL detection system (Amersham-Pharmacia, Arlington Heights, IL). For densitometric analysis of the signals obtained by Western blot were using the ID-image analysis software Kodak digital system. These results were expressed as arbitrary units (a.u.).

2.9. Caspase-3 activity

For caspase-3 activity the CaspGLOW Fluorescein active caspase-3 staining kit (Medical & Biological Laboratories Co., Ltd.) was used following manufacturer instruction. The samples were immediately analyzed by flow cytometry by using FL-1 channel.

2.10. Morphometric analyses

Quantitative evaluation of apoptotic cells and p53 nuclear translocation was performed counting at least 300 cells at high magnification (500×) at the fluorescence microscope.

2.11. Statistical analyses

Cytofluorimetric results were statistically analyzed by using the parametric Kolmogorov–Smirnov test using Cell Quest Software. Morphometric data (reported as means ± standard error, S.E. from at least four separate experiments) were analyzed by using Student's *t*-test. Only $P < 0.05$ was considered as significant.

3. Results and discussion

Photoaging is an extrinsic aging of the skin mainly due to UV-induced damage. UVB radiation crosses the epidermis and interacts with cellular chromophores and photosensitizers, resulting in the generation of reactive oxygen species (ROS), DNA damage (e.g. pyrimidine dimers) and activation of cytoplasmic signal transduction pathways that are related to differentiation, replicative senescence and connective tissue degradation [17]. In the present work, we developed a new model of premature senescence exposing human fibroblasts

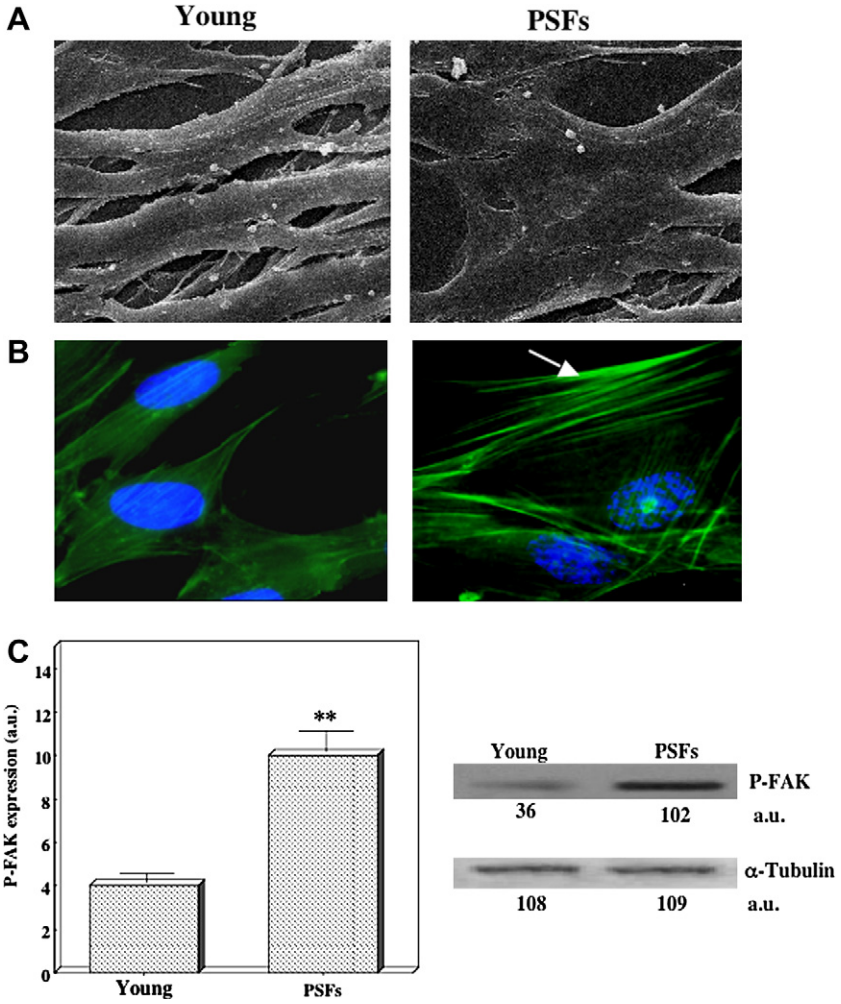

Fig. 2. (A) Young control fibroblasts showing a typical polarized shape (left panel). After UVB irradiation (48 h), prematurely senescent fibroblasts (PSFs) appeared flattened on the cell substrate (right panel). (B) In control cells (left panel) F-actin appears organized in thin and oriented stress fibers through the cell cytoplasm. UVB radiation induced a microfilament system re-organization (right panel), i.e. a thickening of the stress fibers (arrow). (C) Quantitative analyses of P-FAK by flow cytometry (left panel) and Western blot analysis (right panel). Flow cytometry data are reported as mean values ± S.D. from four independent experiments. Student's *t*-test to correlate samples was used. (**) Indicates $P < 0.01$. The values of Western blot signals were obtained by densitometric analysis using the ID-image analysis software Kodak digital system. The results were expressed as arbitrary units (a.u.). Both methodological approaches revealed significant differences between control cells and PSFs.

(WI-38) at early passages to a single sub-cytotoxic dose of UVB radiation (200 mJ/cm^2).

3.1. Cell proliferation

In line with the literature data [1,2], after the 11th passage, more than 40% of WI-38 cells were positive for β-galactosidase, indicating the acquisition of spontaneous senescence, which is referred to as replicative senescence (here labeled as *old*). By contrast, when cells (passages 2–4) were exposed one time to UVB (200 mJ/cm^2) a significant slowing in cell growth was observed. In fact, analysis of growth-curves, obtained as reported in Section 2, demonstrated a time-dependent decrease of the cell number in UVB-irradiated cells with respect to control cells (here labeled as *young*). This started 24 h after irradiation ($-19 \pm 0.6\%$ vs. untreated cells) and became more evident after 48 h ($-24.0 \pm 2.0\%$), after 72 h ($-26.2 \pm 2.3\%$) and five days after UVB irradiation ($-64.5 \pm 5.2\%$). Accordingly, flow cytometry cell cycle analyses also indicated that the percentage of cells in G0/G1 phase was significantly enhanced ($81.3 \pm 8.6\%$) 48 h after UVB irradiation (Fig. 1A, middle panel; PSFs: prematurely senescent fibroblasts) with respect to young fibroblasts (Fig. 1A, left panel). In spontaneously senescent fibroblasts (11th passage), the percentage of cells in G0/G1 was found very similar to UVB-irradiated cells (Fig. 1A, right panel).

3.2. β-Galactosidase activity

Considering that an increase of β-galactosidase activity is generally recognized as a typical biomarker of replicative senescence [18,19], analyses were carried out by using a specific detection assay for this enzyme. These analyses clearly indicated that, with respect to young cells, the number of β-galactosidase-positive cells increased with time after radiation. After 72 h the percentage of β-galactosidase PSFs was similar to that detected in cells undergoing replicative senescence (Fig. 1B).

3.3. Cell morphology and cytoskeleton remodeling

Morphological analyses of UVB exposed cells clearly showed an enlarged and flattened cell phenotype (Fig. 2A) as detected by scanning electron microscopy and a remodeling of cytoskeleton (Fig. 2B). In particular, we focused our attention on the main cytoskeletal protein responsible for cell shape and polarity maintenance [20] as well as for the maintenance of cell-to-cell and cell-substrate interactions, i.e. microfilamentous F-actin [21]. We observed that, after UVB exposure, a marked cytoskeleton reorganization clearly occurred. In fact, while in young cells the actin microfilaments appeared organized in thin and oriented stress fibers throughout the cell cytoplasm (Fig. 2B, left panel), in PSFs a dramatic redistribution and "thickening" of the actin stress fibers was detectable (Fig. 2B, right panel). Furthermore, we analyzed the expression of the focal adhesion kinase (FAK) protein, which is involved in cell-substrate interaction [22]. In particular, we evaluated the expression of phosphorylated (activated) form (P-FAK) of this protein by using both flow cytometry and Western blot analysis. As summarized in Fig. 2C (left panel, where the mean values ± S.D. from four independent experiments performed by flow cytometry are shown), the expression level of P-FAK increased significantly ($P < 0.01$) 48 h after UVB exposure with respect to control cells. These data were also confirmed by Western blotting analysis (right panel, numbers represent densitometric analyses reported as arbitrary units, a.u.).

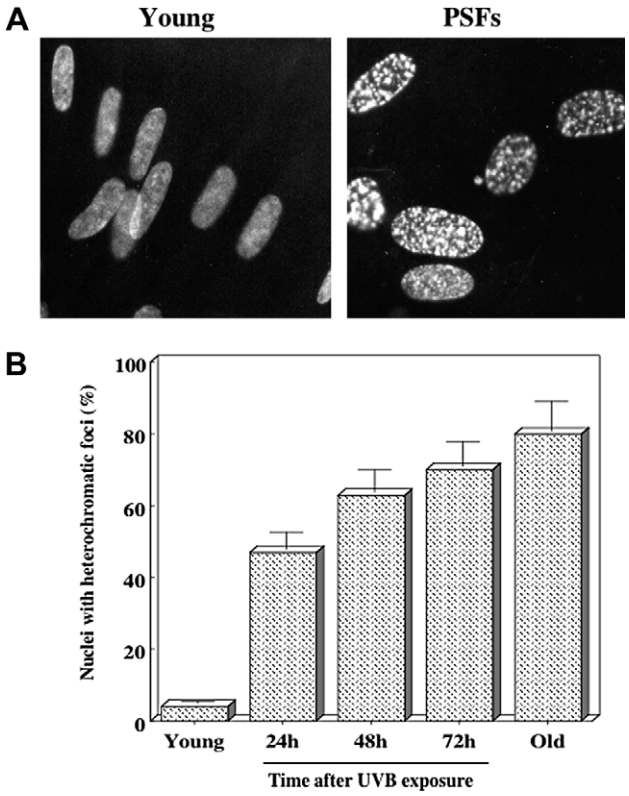

Fig. 3. (A) Fluorescence microscopy of WI-38 control cells (left panel) and prematurely senescent fibroblasts (PSFs) 48 h after UVB exposure (right panel) stained with Hoechst 33258. Note the formation of heterochromatin foci in PSFs. (B) Morphometric analysis indicating the percentage of nuclei with heterochromatic foci in young and old fibroblasts at different time points after UVB irradiation. Mean values from four independent experiments ± S.D. are shown. Note as the percentage of nuclei with heterochromatic foci increases with increasing time after UVB exposure.

3.4. Nuclear morphology

To further characterize chromatin changes associated to cellular senescence, we analyzed the nuclei of PSFs by using a specific DNA staining dye (Hoechst staining). No signs of nuclear damage or apoptosis, i.e. chromatin condensation or fragmentation, were visible 48 h after UVB exposure, but, as

Table 1
Evaluation of reactive oxygen species by flow cytometry analysis after UVB irradiation

	30 min	1 h	3 h
$O_2^{\cdot}$	+39.3 ± 3.1*	+42.5 ± 6.2*	−4.3 ± 2.1
H_2O_2	+5.4 ± 2.0	−1.2 ± 0.4	−5.1 ± 3.3
GSH	+15.1 ± 4.3*	−5.2 ± 2.0	−12.7 ± 3.1*

Flow cytometry analysis of ROS production and intracellular GSH level. The median values of fluorescence intensity histograms were used to provide semi-quantitative analysis. The fluorescence signal of control fibroblasts was set to 100% and the expression levels of each parameter after UVB irradiation are reported as relative to this. Numbers represent the means ± S.D. from three independent experiments.
*Indicates $P < 0.01$.

shown in Fig. 3A (right panel), PSFs underwent a peculiar reorganization of nuclear chromatin with respect to control cells (Fig. 3A, left panel). In particular, distinct heterochromatic foci, typical of cell senescence and detectable as a scattered fluorescent spots, were detected in the nuclei of PSFs. Moreover, a quantification of cells with heterochromatic foci was performed at different time points after UVB exposure (Fig. 3B). Notably, we found that after 72 h, the percentage of cells with heterochromatic foci was very similar in PSFs and in old cells, i.e. in fibroblasts in replicative senescence.

3.5. Redox balance

It is well known that "intermediate amounts" of reactive oxygen species (ROS) are able to induce permanent growth arrest and replicative senescence [23]. In light of the above results, we carried out specific time course experiments in order to verify if ROS production could be involved in UVB-induced premature senescence of WI-38 fibroblasts. In particular, the cellular redox state, monitored by flow cytometry analysis by using specific probes as indicated in Section 2, was evaluated after 30 min, 1 h and 3 h from the UVB irradiation. What we found can be summarized as follows: (i) 30 min and 1 h after UVB irradiation we observed a significant increase ($P < 0.01$) of superoxide anion ($O_2^{\cdot}$) generation (30 min: +39 ± 9%; 1 h: +42 ± 10%) with respect to untreated fibroblasts. By contrast, (ii) 3 h after irradiation no differences in term of superoxide anion production were detected in both treated and untreated cells ($P > 0.05$). Moreover, (iii) UVB radiation did not induce any significant alteration in terms of H_2O_2 production. Finally, (iv) a significant increase of GSH (+15% ± 4.3) at early time points (e.g. 30 min after irradiation) with respect to control cells was observed. Interestingly, at

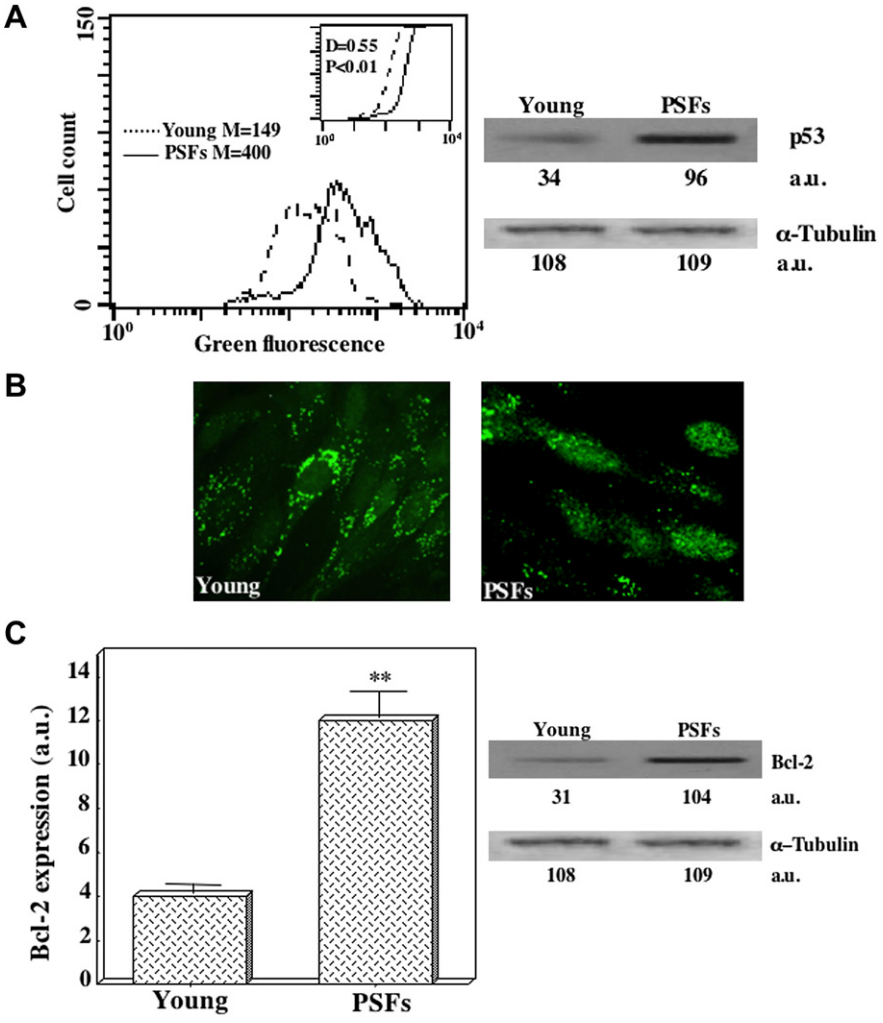

Fig. 4. (A) Quantitative and (B) qualitative analysis of p53 as detected by flow cytometry, Western blot, and IVM, respectively. Note the increased expression of p53 in prematurely senescent fibroblasts (PSFs) (A, left panel, black line) with respect to control cells (A, left panel, dotted line) as revealed by flow cytometry. Numbers represent median values of fluorescence intensity histograms. Statistical analysis was performed by using the Kolmogorov–Smirnov test (inset). One representative experiment of four is shown. Western blot analysis confirms the increase of p53 in PSFs with respect to control cells (A, right panel). The values of Western blot signals (expressed as arbitrary units, a.u.) were obtained by densitometric analysis as stated above. (B) IVM analysis showing the translocation into the nucleus of p53 in PSFs (B, right panel) with respect to control fibroblasts (B, left panel) where this protein appears randomly distributed through the cell cytoplasm. (C) Quantitative analysis of Bcl-2 protein by flow cytometry (C, left panel) and Western blot (C, right panel). Flow cytometry data are reported as mean values ± S.D. from four independent experiments. In ordinate, median fluorescence intensity is reported. Student's *t*-test to correlate samples was used. (**) Indicates $P < 0.01$. The values of Western blot signals were obtained by densitometric analysis and expressed as arbitrary units (a.u.).

later time points, the levels of GSH stably and significantly decreased in UVB irradiated fibroblasts (1 h: −5.2% ± 2.0; 3 h: −12.7% ± 3.1) when compared with control young fibroblasts. The results obtained at different time points after UVB exposure are reported in Table 1.

3.6. Expression and localization of p53

Considering that ROS are potent activators of p53 signaling molecule [24] and that p53 expression was suggested to play a critical role in determining cell fate, i.e. in terms of senescence or apoptosis [25], we investigated about the possible role of p53 in our senescence cell system. As shown in Fig. 4A, 3 h after UVB exposure a significant increase of this protein was observed by both flow cytometry (left panel) and Western blotting (right panel). Moreover, localization of p53 was also investigated by fluorescence microscopy analysis (Fig. 4B). Interestingly, 3 h after UVB exposure, p53 molecule translocated from the cytoplasm (Fig. 4B, left panel, control cells) into the nucleus (Fig. 4B, right panel, PSFs) where it is known to participate to the control of cell cycle [26].

3.7. Analysis of the anti-apoptotic protein Bcl-2

The ability of a cell to survive after an insult can depend upon the modulation of a plethora of proteins [27,28], among these there are Bcl-2 family proteins. Thus, we analyzed the expression of Bcl-2, an anti-apoptotic protein mainly located on the outer membrane of mitochondria that, when overexpressed, prevents cells from undergoing apoptosis in response to a variety of stimuli [9]. Either flow cytometry (Fig. 4C, left panel) or Western blot (Fig. 4C, right panel) analysis clearly demonstrated a significant increase of Bcl-2 protein in fibroblasts 48 h after UVB irradiation ($P < 0.01$ with respect to control cells).

It is well known that a sustained oxidative stress is associated to apoptosis and activation of caspases [27]. By contrast, in our conditions, a transient and mild oxidative imbalance was detected without any sign of caspase activation (data not shown) and apoptosis. Accordingly, anti-apoptotic molecule Bcl-2 was found up-regulated in UVB irradiated cells. In line with previous findings [18,27], this argues in favor of a signaling role exerted by ROS. The up-regulation of ROS production might be related to retrograde response initiated by mitochondria and could involve signals from the p53 pathway. Conceivably, as for other senescence systems [3,4,18,23], this could also depend on the activation of stress kinases, such as p38 (manuscript in preparation) and could be reverted by antioxidant treatments [18]. Fittingly, focal adhesion formation, i.e. FAK phosphorylation and cell spreading (see Fig. 2), are significantly activated by redox signaling [29]. Hence, the cascade of events leading to premature senescence induced by a single exposure to UVB radiation seems to be instructed by a specific ROS signaling pathway that could be analyzed in detail in future studies.

Notwithstanding, it has already been demonstrated that several and repeated exposures to DNA damaging agents or a variety of oxidative stress, including exposures to UV radiation given at non-cytotoxic (non-apoptotic) doses, are capable of inducing premature senescence [18]. Thus, the novelty of our system resides on the single exposure of UVB radiation we used to induce premature senescence. This simple and rapid method can in fact provide an experimental tool bypassing the "intrinsic" replicative senescence due to repeated passages and treatments. Thus, we propose this model as an alternative in vitro experimental system useful in photoaging research as well as in testing potential anti-photoaging compounds.

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References

[1] Campisi, J. (1996) Senescence: an old lives' tale? Cell 84, 497–500.
[2] Campisi, J. (2001) From cells to organisms: can we learn about aging from cells in culture? Exp. Gerontol. 36, 607–618.
[3] Robles, S.J. and Adami, G.R. (1998) Agents that cause DNA double strand breaks lead to p16INK4a enrichment and the premature senescence of normal fibroblasts. Oncogene 16, 1113–1123.
[4] Serrano, M. and Blasco, M.A. (2001) Putting the stress on senescence. Curr. Opin. Cell Biol. 13, 748–753.
[5] Serrano, M., Lin, A.W., McCurrach, M.E., Beach, D. and Lowe, S.W. (1997) Oncogenic ras provokes premature cell senescence associated with accumulation of p53 and p16INK4a. Cell 88, 593–602.
[6] Dimri, G.P., Lee, X., Basile, G., Acosta, M., Scott, G., Roskelley, C., Medrano, E.E., Linskens, M., Rubelj, I. and Pereira-Smith, O. (1995) A biomarker that identifies senescent human cells in culture and in aging skin in vivo. Proc. Natl. Acad. Sci. 92, 9363–9367.
[7] Petersen, S., Saretzki, G. and von Zglinicki, T. (1998) Preferential accumulation of single-stranded regions in telomeres of human fibroblasts. Exp. Cell Res. 239, 152–160.
[8] Rosette, C. and Karin, M. (1996) Ultraviolet light and osmotic stress: activation of the JNK cascade through multiple growth factor and cytokine receptors. Science 274, 1194–1197.
[9] Helenius, M., Makelainen, L. and Salminen, A. (1999) Attenuation of NF-kB signaling response to UVB light during cellular senescence. Exp. Cell Res. 248, 194–202.
[10] Straface, E., Giacomoni, P.U. and Malorni, W. (2001) Cultured cells as a model system for the study of UV-induced cytotoxicity. J. Photochem. Photobiol. 63, 52–60.
[11] Gasparro, F.G. and Brown, D.B. (2000) UV sources and dosimetry-the proper use and measurement of "photons as a reagent". J. Invest. Dermatol. 114, 613–615.
[12] Parlato, S., Giammarioli, A.M., Logozzi, M., Lozupone, F., Matarrese, P., Luciani, F., Falchi, M., Malorni, W. and Fais, S. (2000) CD95 (APO-1/Fas) linkage to the actin cytoskeleton through ezrin in human T lymphocytes: a novel regulatory mechanism of the CD95 apoptotic pathway. EMBO J. 19, 5123–5134.
[13] Lugini, L., Matarrese, P., Tinari, A., Lozupone, F., Federici, C., Iessi, E., Gentile, M., Luciani, F., Parmiani, G., Rivoltini, L., Malorni, W. and Fais, S. (2006) Cannibalism of live lymphocytes by human metastatic but not primary melanoma cells. Cancer Res. 66, 3629–3638.
[14] Straface, E., Santini, M.T., Donelli, G., Giacomoni, P.L. and Malorni, W. (1995) Vitamin E prevents UVB-induced cell blebbing and cell death in A431 epidermoid cells. Int. J. Radiat. Biol. 68, 579–587.
[15] Frey, T. (1997) Correlated flow cytometric analysis of terminal events in apoptosis reveals the absence of some changes in some model systems. Cytometry 28, 253–263.
[16] Sebastia, J., Cristofol, R., Martin, M., Rodriguez-Farre, E. and Sanfeliu, C. (2003) Evaluation of fluorescent dyes for measuring intracellular glutathione content in primary cultures of human neurons and neuroblastoma SH-SY5Y. Cytometry 51, 16–25.
[17] Wlaschek, M., Ma, W., Jansen-Durr, P. and Scharffetter-Kochanek, K. (2003) Photoaging as a consequence of natural and therapeutic ultraviolet irradiation–studies on PUVA-induced senescence-like growth arrest of human dermal fibroblasts. Exp. Gerontol. 38, 1265–1270.
[18] Debacq-Chainiaux, F., Borlon, C., Pascal, T., Royer, V., Eliaers, F., Ninane, N., Carrard, G., Friguet, B., de Longueville, F., Boffe, S., Remacle, J. and Toussaint, O. (2005) Repeated exposure of human skin fibroblasts to UVB at subcytotoxic level triggers premature senescence through the TGF-{beta}1 signaling pathway. J. Cell Sci. 118, 743–747.

[19] Maier, A.B., Westendorp, R.G. and VAN Heemst, D. (2007) Beta-galactosidase activity as a biomarker of replicative senescence during the course of human fibroblast cultures. Ann. NY Acad. Sci. 1100, 323–332.
[20] Fais, S. and Malorni, W. (2003) Leukocyte uropod formation and membrane/cytoskeleton linkage in immune interactions. J. Leukoc. Biol. 73, 556–563.
[21] Webb, D.J., Parsons, J.T. and Horwitz, A.F. (2002) Adhesion assembly, disassembly and turnover in migrating cells-over and over again. Nat. Cell Biol. 4, E97–E100.
[22] Huang, D., Khoe, M., Befekadu, M., Chung, S., Takata, Y., Ilic, D. and Bryer-Ash, M. (2007) Focal adhesion kinase mediates cell survival via NF-kappaB and ERK signaling pathways. Am. J. Physiol. Cell Physiol. 292, C1339–C1352.
[23] Martindale, J.L. and Holbrook, N.J. (2002) Cellular response to oxidative stress: signaling for suicide and survival. J. Cell Physiol. 192, 1–15.
[24] Macip, S., Igarashi, M., Berggren, P., Yu, J., Lee, S.W. and Aaronson, S.A. (2003) Influence of induced reactive oxygen species in p53-mediated cell fate decisions. Mol. Cell Biol. 23, 8576–8585.
[25] Cotton, J. and Spandau, D.F. (1997) Ultraviolet B-radiation dose influences the induction of apoptosis and p53 in human keratinocytes. Radiat. Res. 147, 148–155.
[26] Assefa, Z., Van Laethem, A., Garmyn, M. and Agostinis, P. (2005) Ultraviolet radiation-induced apoptosis in keratinocytes: on the role of cytosolic factors. Biochim. Biophys. Acta 1755, 90–106.
[27] Chandra, J., Samali, A. and Orrenius, S. (2000) Triggering and modulation of apoptosis by oxidative stress. Free Radic. Biol. Med. 29, 323–333.
[28] Maddika, S., Ande, S.R., Panigrahi, S., Paranjothy, T., Weglarczyk, K., Zuse, A., Eshraghi, M., Manda, K.D., Wiechec, E. and Los, M. (2007) Cell survival, cell death and cell cycle pathways are interconnected: implications for cancer therapy. Drug Resist. Updat. 10, 13–29.
[29] Chiarugi, P., Pani, G., Giannoni, E., Taddei, L., Colavitti, R., Raugei, G., Symons, M., Borrello, S., Galeotti, T. and Ramponi, G. (2003) Reactive oxygen species as essential mediators of cell adhesion: the oxidative inhibition of a FAK tyrosine phosphatase is required for cell adhesion. J. Cell Biol. 161, 933–944.